EXHIBIT A

GOODMAN & GILMAN'S The PHARMACOLOGICAL BASIS OF THERAPEUTICS

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CHAPTER 60

INSULIN, ORAL HYPOGLYCEMIC AGENTS, AND THE PHARMACOLOGY OF THE ENDOCRINE PANCREAS

Stephen N. Davis and Daryl K. Granner

This chapter provides background on the pharmacological actions of insulin, glucagon, somatostatin, and hypoglycemic agents. The discovery of insulin in 1921 allowed the previously fatal disorder of insulin-dependent diabetes mellitus to be treated and represented a landmark in medical history. In the first part of this chapter, the diverse physiological functions of insulin are described at the cellular and whole-body level. This section establishes the role of insulin in the treatment of diabetes mellitus. The next section describes the pharmacodynamics and pharmacokinetics of exogenously administered insulin and highlights the benefits of intensive insulin therapy in limiting long-term tissue complications of diabetes. The chapter continues with descriptions of the pharmacology of oral hypoglycemic and antihyperglycemic agents. These agents have an important role in the treatment of non-insulin-dependent diabetes mellitus, the most common form of diabetes. This section also covers some of the newer blood glucose-lowering agents currently undergoing clinical trials. The final part of the chapter describes the physiology and pharmacology of glucagon and somatostatin, with emphasis on the expanding use of somatostatin analogs in clinical medicine.

INSULIN

History. Few events in the history of medicine are more dramatic than the discovery of insulin. Although the discovery is appropriately attributed to Banting and Best, several other investigators and collaborators provided important observations and techniques that made it possible. In 1869, a German medical student, Paul Langerhans, noted that the pancreas contains two distinct groups of cells—the acinar cells, which secrete digestive enzymes, and cells that are clustered in islands, or islets, which he suggested served a second function. Direct evidence for this function came in 1889, when Oskar Minkowski and Joseph von Mering showed that pancreatectomized dogs exhibit a syndrome similar to diabetes mellitus in human beings (*see* Minkowski, 1989).

There were numerous attempts to extract the pancreatic substance responsible for regulating blood glucose. In the early 1900s, Gurg Ludwig Zuelzer, an internist in Berlin, attempted to treat a dying diabetic patient with extracts of pancreas. Although the patient improved temporarily, he sank back into coma and died when the supply of extract was exhausted. E. L. Scott, a student at the University of Chicago, made another early attempt to isolate an active principle in 1911. Using alcoholic extracts of the pancreas (not so different from those eventually used by Banting and Best), Scott treated several diabetic dogs with encouraging results; however, he lacked clear measures of control of blood glucose concentrations, and his professor considered the experiments inconclusive at best. Between 1916 and 1920, the Romanian physiologist Nicolas Paulesco conducted a series of experiments in which he found that injections of pancreatic extracts reduced urinary sugar and ketones in diabetic dogs. Although he published the results of his experiments, their significance was fully appreciated only many years later.

Unaware of much of this previous work, in 1921 Frederick G. Banting, a young Canadian surgeon, convinced a professor of physiology in Toronto, J. J. R. Macleod, to allow him access to a laboratory to search for the antidiabetic principle of the pancreas. Banting assumed that the islet tissues secreted insulin, but that the hormone was destroyed by proteolytic digestion prior to or during extraction. Together with a fourth-year medical student, Charles H. Best, he attempted to overcome the problem by tying the pancreatic ducts. The acinar tissue degenerated, leaving the islets undisturbed; the remaining tissue was then extracted with ethanol and acid. Banting and Best thus obtained a pancreatic extract that was effective in decreasing the concentration of blood glucose in diabetic dogs.

The first patient to receive the active extracts prepared by Banting and Best was Leonard Thompson, aged 14 (Banting *et al.*, 1922). He appeared at the Toronto General Hospital with a blood glucose of 500 mg/dl (28 mM), and he was excreting 3 to 5 liters of urine per day. Despite rigid control of diet (450 kcal per day), he continued to excrete large quantities of glucose, and, without insulin, the most likely outcome was death after a few months. The administra-

usually well tolerated. Smaller doses are given with snacks. Acarbose is most effective when given with a starchy, high-fiber diet with restricted amounts of glucose and sucrose (Bressler and Johnson, 1992).

GLUCAGON

History. Distinct populations of cells were identified in the islets of Langerhans before the discovery of insulin. Glucagon itself was discovered by Murlin and Kimball in 1923, less than 2 years after the discovery of insulin. In contrast to the excitement caused by the discovery of insulin, few were interested in glucagon, and it was not recognized as an important hormone for over 40 years. Glucagon is now known to have a significant physiological role in the regulation of glucose and ketone body metabolism, but it is only of minor therapeutic interest for the short-term management of hypoglycemia. It is also used in radiology for its inhibitory effects on intestinal smooth muscle.

Chemistry. Glucagon is a 29-amino-acid, single-chain polypeptide (Figure 60–6). It shows significant homology with several other polypeptide hormones, including secretin, vasoactive intestinal peptide, and gastrointestinal inhibitory polypeptide. The primary sequence of glucagon is highly conserved in mammals, and it is identical in human beings, cattle, pigs, and rats.

Glucagon is synthesized from preproglucagon, a 180-amino-acid precursor with five separately processed domains (Bell *et al.,* 1983). An amino-terminal signal peptide is followed by glicentin-related pancreatic peptide, glucagon, glucagon-like peptide-1, and glucagon-like peptide-2. Processing of the protein is sequential and occurs in a tissue-specific fashion; this results in different secretory peptides in pancreatic α cells and intestinal α-like cells (termed *L cells*) (Mojsov *et al.,* 1986). Glicentin, a major processing intermediate, consists of glicentin-related pancreatic polypeptide at the amino terminus and glucagon at the carboxyl terminus, with an Arg-Arg pair between. Enteroglucagon (or oxyntomodulin) consists of glucagon and a carboxyl-terminal hexapeptide linked by an Arg-Arg pair.

The biological roles of these precursor peptides are uncertain, but the highly controlled nature of the processing suggests that these peptides may have distinct biological functions. In the pancreatic α cell, the granule consists of a central core of glucagon surrounded by a halo of glicentin. Intestinal L cells contain only glicentin and presumably lack the enzyme required to process this precursor to glucagon. Enteroglucagon binds to hepatic glucagon receptors and stimulates adenylyl cyclase with 10 to 20% of the potency of glucagon. Glucagon-like peptide-1 is an extremely potent potentiator of insulin secretion, although it apparently lacks significant hepatic actions. Glicentin, enteroglucagon, and the glucagon-like peptides are found predominantly in the intestine, and their secretion continues after total pancreatectomy.

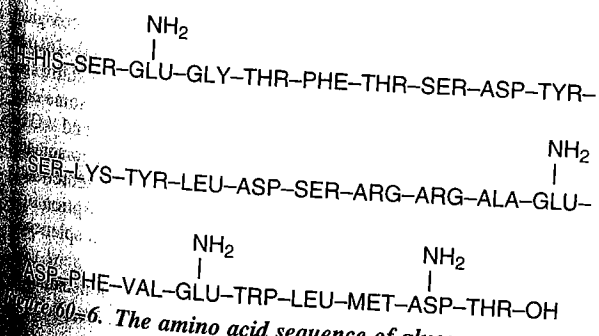

Figure 60–6. The amino acid sequence of glucagon.

Regulation of Secretion. The secretion of glucagon is regulated by dietary glucose, insulin, amino acids, and fatty acids; glucose is a potent inhibitor. As in insulin secretion, glucose is a more effective inhibitor of glucagon secretion when taken orally than when administered intravenously, suggesting a possible role for some gastrointestinal hormone in the response. The effect of glucose is lost in the untreated or undertreated IDDM patient and in isolated pancreatic α cells, indicating that at least part of the effect is secondary to stimulation of insulin secretion. Somatostatin also inhibits glucagon secretion, as do free fatty acids and ketones.

Most amino acids stimulate the release of both glucagon and insulin. This coordinated response to amino acids may prevent insulin-induced hypoglycemia in individuals who ingest a meal of pure protein. Like glucose, amino acids are more potent when taken orally and thus may exert some of their effects via gastrointestinal hormones. Secretion of glucagon also is regulated by the autonomic innervation of the pancreatic islet. Stimulation of sympathetic nerves or administration of sympathomimetic amines increases glucagon secretion. Acetylcholine has a similar effect.

Glucagon in Diabetes Mellitus. Plasma concentrations of glucagon are elevated in poorly controlled diabetic patients. In view of its capacity to enhance gluconeogenesis and glycogenolysis, glucagon exacerbates the hyperglycemia of diabetes. However, this abnormality of glucagon secretion appears to be secondary to the diabetic state and is corrected with improved control of the disease (Unger, 1985). The importance of the hyperglucagonemia in diabetes has been evaluated by administration of somatostatin (Gerich *et al.,* 1975). Although somatostatin does not restore glucose metabolism to normal, it significantly slows the rate of development of hyperglycemia and ketonemia in insulinopenic IDDM subjects. In normal individuals, glucagon secretion increases in response to hypoglycemia, but in IDDM patients this important defense mechanism (against insulin-induced hypoglycemia) is lost early in the course of the disease.

Degradation. Glucagon is extensively degraded in liver, kidney, and plasma, as well as at its sites of action (Peterson *et al.,* 1982). Its half-life in plasma is approximately 3 to 6 minutes. Proteolytic removal of the amino-terminal histidine residue leads to loss of biological activity.

Cellular and Physiological Actions. Glucagon interacts with a 60-kDa glycoprotein receptor on the plasma membrane of target cells (Sheetz and Tager, 1988). Although the exact structure of this receptor is not yet known, it interacts with the stimulatory guanine-nucleotide-binding regulatory protein, G_s, that activates adenylyl cyclase (*see* Chapter 2). The primary effects of glucagon on the liver are mediated by cyclic AMP. In general, modifications of the amino-terminal region of glucagon (*e.g.,* [Phe^1]glucagon and des-His$_1$-[Glu^9]glucagon amide) result in molecules that behave as partial agonists—they retain some affinity for the glucagon receptor but have a markedly reduced capacity to stimulate adenylyl cyclase (Unson *et al.,* 1989).

Phosphorylase, the rate-limiting enzyme in glycogenolysis, is activated by glucagon as a result of cyclic AMP-stimulated phosphorylation, while concurrent phosphorylation of glycogen synthase inactivates the enzyme; glycogenolysis is enhanced and glycogen synthesis is inhibited. Cyclic AMP also stimulates transcription of the gene for phosphoenolpyruvate carboxykinase, a rate-limiting enzyme in gluconeogenesis (Granner *et al.,* 1986). These effects are normally opposed by insulin, and when equivalent equations of both hormones are present, insulin is dominant.

Cyclic AMP also stimulates phosphorylation of the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (Pilkis *et al.,* 1981; Foster, 1984). This enzyme determines the cellular concentration of fructose-2,6-bisphosphate, which acts as a potent regulator of gluconeogenesis and glycogenolysis. When the concentration of glucagon is high relative to that of insulin, this enzyme is phosphorylated and acts as a phosphatase, reducing the concentration of fructose-2,6-bisphosphate in the liver. When the concentration of insulin is high relative to that of glucagon, the enzyme is dephosphorylated and acts as a kinase, raising fructose-2,6-bisphosphate concentrations. Fructose-2,6-bisphosphate interacts allosterically with phosphofructokinase-1, the rate-limiting enzyme in glycolysis, increasing its activity. Thus, when glucagon concentrations are high, glycolysis is inhibited and gluconeogenesis is stimulated. This also leads to a decrease in the concentration of malonyl CoA, stimulation of fatty acid oxidation, and production of ketone bodies. Conversely, when insulin concentrations are high, glycolysis is stimulated and gluconeogenesis and ketogenesis are inhibited (*see* Foster, 1984).

Glucagon exerts effects on tissues other than liver, especially at higher concentrations. In adipose tissue, it stimulates adenylyl cyclase and increases lipolysis. In the heart, glucagon increases the force of contraction. Glucagon has relaxant effects on the gastrointestinal tract; this has been observed with analogs that apparently do not stimulate adenylyl cyclase. Some tissues (including liver) possess a second type of glucagon receptor that is linked to generation of inositol trisphosphate, diacylglycerol, and Ca^{2+} (Murphy *et al.,* 1987). The role of this receptor in regulation of metabolism remains uncertain.

Therapeutic Use. Glucagon is used to treat severe hypoglycemia, particularly in diabetic patients when intravenous glucose is not available; it also is used by radiologists for its inhibitory effects on the gastrointestinal tract.

All glucagon used clinically is extracted from bovine and porcine pancreas; its sequence is identical to that of the human hormone. For hypoglycemic reactions, 1 mg is administered intravenously, intramuscularly, or subcutaneously. Either of the first two routes is preferred in an emergency. Clinical improvement is sought within 10 minutes to minimize the risk of neurological damage from hypoglycemia. The hyperglycemic action of glucagon is transient and may be inadequate if hepatic stores of glycogen are depleted. After the initial response to glucagon, patients should be given glucose or urged to eat to prevent recurrent hypoglycemia. Nausea and vomiting are the most frequent adverse effects.

Glucagon also is used to relax the intestinal tract to facilitate radiographic examination of the upper and lower gastrointestinal tract with barium and retrograde ileography (Monsein *et al.,* 1986) and in magnetic resonance imaging of the gastrointestinal tract (Goldberg and Thoeni, 1989). Glucagon has been used to treat the spasm associated with acute diverticulitis and disorders of the biliary tract and sphincter of Oddi, as an adjunct in basket retrieval of biliary calculi, and for impaction of the esophagus and intussusception (Friedland, 1983; Mortensson *et al.,* 1984; Kadir and Gadacz, 1987). It has been used for diagnostic purposes to distinguish obstructive from hepatocellular jaundice (Berstock *et al.,* 1982).

Glucagon releases catecholamines from a pheochromocytoma and has been used experimentally as a diagnostic test for this disorder. The hormone also has been used as a cardiac inotropic agent for the treatment of shock, particularly when prior administration of a β-adrenergic receptor antagonist has rendered β-adrenergic receptor agonists ineffective.

SOMATOSTATIN

Somatostatin was first isolated and synthesized in 1973, following a search for hypothalamic factors that might regulate secretion of growth hormone from the pituitary gland (Brazeau *et al.,* 1973; *see* also Chapter 55). A potential physiologic role for somatostatin in the islet was suggested by the observation that somatostatin inhibits secretion of insulin and glucagon (Alberti *et al.,* 1973; Gerich *et al.,* 1974). The peptide subsequently was identified in the D cells of the pancreatic islet, in similar cells of the gastrointestinal tract, and in the central nervous system (Dubois, 1975).

Somatostatin, the name originally given to a cyclic peptide containing 14 amino acids, is now known to be one of a group of related peptides. These include the original somatostatin (S-14), an extended 28-amino-acid peptide molecule (S-28), and a fragment containing the initial 12 amino acids of somatostatin-28 (S-28[1-12]). Somatostatin-14 is the predominant form in the brain, whereas somatostatin-28 is the main form in the gut. Somatostatin inhibits the release of thyroid stimulating hormone and growth hormone from the pituitary gland, of gastrin, motilin, vasoactive intestinal peptide (VIP), glicentin, and gastrointestinal polypeptide from the gut, and of insulin, glucagon pancreatic polypeptide and somatostatin from the pancreas.

Somatostatin secreted from the pancreas can regulate pituitary function, thereby acting as a true neurohormone. In the gut, however, somatostatin acts as a paracrine agent by influencing the functions of adjacent cells. It also can act as an autocrine agent by inhibiting its own release at the pancreas. The D cell is the last to receive blood flow in the islet; that is, it is downstream from the β and α cells (Samols *et al.,* 1986). Thus, somatostatin may regulate the secretion of insulin and glucagon only via the systemic circulation.

Somatostatin is released in response to many of the nutrients and hormones that stimulate insulin secretion, including glucose, arginine, leucine, glucagon, vasoactive intestinal polypeptide, cholycystokinin, and even tolbutamide (Ipp *et al.,* 1977; Weir *et al.,* 1979). The physiological role of somatostatin has not been precisely defined. When administered in pharmacological amounts, somatostatin inhibits virtually all endocrine and exocrine secretions of the pancreas, gut, and gall bladder. Somatostatin also can inhibit secretion of the salivary glands and, under some conditions, can block parathyroid, calcitonin, prolactin, and ACTH secretion. The α cell is about 50 times more sensitive to somatostatin than is the β cell, but inhibition of glucagon secretion is more transient. Somatostatin also inhibits nutrient absorption from the intestine, decreases intestinal motility, and reduces splanchnic blood flow.

The therapeutic uses of somatostatin are confined mainly to blocking hormone release in endocrine-secreting tumors, including